P53 MUTATIONS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma that is characterized by an aggressive clinical course. Aberrations of bcl-2, bcl-6 and c-myc genes are common markers of this disease. Mutations in the p53 gene occurring in many tumors are also frequent in DLBCL where they are associated with poor response to treatment and short survival.

In our study, the p53 status was analyzed in tumor tissue of 127 patients with diffuse large B-cell lymphoma. Several methods have been used. For study of p53 transcriptional activity we used functional assay in yeast – FASAY. Mutations in the p53 gene were determined by cDNA sequencing. Level of the p53 protein was assessed by immunoblotting and the loss of heterozygosity of p53 was analyzed by interphase FISH.

We detected 25 p53 mutations (19.7%) in 25 cases of DLBCL. Two mutations showed up repeatedly (p.R273H, p.Q136E) and two independent missense mutations were detected in two cases. Twenty mutations were found in de novo DLBCL patients (17.9%), five in transformed cases (21.7%). Most of the mutations – 22 - were missense (88.0%). Five of them were temperature-sensitive (22.7%) what exceeded the average incidence. We identified two mutations causing formation of premature termination codon. Non-sense mutation in codon 196 induced non-sense-mediated mRNA decay (NMD) pathway resulting in mRNA degradation; no p53 protein was detected. In contrast, termination codon in position 345 led to expression of low level of truncated p53 protein. In one case, short in frame deletion was detected.
Objective: To compare PET/CT to contemporary subspeciality CT imaging of lymphoma at Mayo Clinic Arizona.

Methods: The authors retrospectively review of all cases of lymphoma evaluated with PET/CT within the most recent 24-month period, which had additional contemporary CT imaging. PET/CT is interpreted by three board certified imagers in both Radiology and Nuclear medicine, and dedicated contrast-enhanced CT is performed by subspecialists of head and neck, and thoracic and abdominal imaging.

Results: Among 111 patients with PET/CT for staging and post-therapeutic monitoring of lymphoma, and additional contemporary dedicated CT evaluation: (1) PET/CT is more informative than CT in 38 cases, (2) PET/CT and CT are equally informative in 71 cases, and (3) PET/CT misses 2 cases of CT-depicted acute findings of pulmonary embolus and non-occlusive venous thrombosis.

For all cases, PET/CT provides a more comprehensive evaluation in the scope of Deauville lymphoma scoring than truncated subspecialty CT interpretation. The superiority of PET/CT over CT also includes findings at CT “blind spots” such as extremities, cutaneous layers, lesions not fulfilling size and enhancing criteria of CT for abnormalities but F-18 FDG-avid on PET, and co-existing independent malignancies of other organs.

Conclusions: At our institution, PET/CT imaging of lymphoma provides adequate staging and post-therapeutic surveillance data for optimal patient care. Additional subspeciality CT imaging is not necessary except for acute processes occurring during the clinical course of lymphoma.
Poster

RELATIONSHIP BETWEEN EXPOSURE TO PESTICIDES AND OCCURRENCE OF LYMPHOID NEOPLASM

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Background: The etiology of malignant lymphoma is still largely unknown. This study determines the relationship between exposure to pesticides and the occurrence of lymphoid neoplasms in Shiraz, Southern Iran.

Methods: Between 2007 and 2008, in a case control study conducted in Nemazee Hospital in Shiraz, Southern Iran, 200 subjects diagnosed with lymphoma according to the World Health Organization (WHO) classification were enrolled. Controls (n=200) were frequency matched to the cases by sex, age, and center. Subjects who were a farmer were compared with all other occupations.

Results: Out of the 200 cases that were diagnosed as lymphoid neoplasms, 100 were non-Hodgkin`s lymphoma, 54 Hodgkin`s lymphoma and 46 multiple myeloma. Seventy two percent of the NHL`s were of the B-cell type, 15% of the T-cell type and the rest were not classified. Furthermore, subjects exposed to pesticides were at an increased risk of non-Hodgkin lymphoma and MM, but not Hodgkin lymphoma.

Conclusion: Risk of non-Hodgkin lymphoma and MM was highest for exposure to pesticides, among them, insecticide`s risk was confirmed.
Hematopoietic stem cell transplantation (HSCT) has been increasingly used as a curative treatment for acute myeloid leukemia (AML). However, relapse rates after HSCT in complete remission (CR) are reported between 30% and 70%. In addition, numerous studies suggested that secondary viral infection from a variety of viruses including Epstein-Barr virus (EBV), adenovirus (Adv), and cytomegalovirus (CMV) is among the most common causes of death post-HSCT. Currently, chimeric antigen receptor (CAR)-based T cells have been developed to treat AML in clinical studies, while virus-specific cytotoxic T cells (VST) have been proven to be able to effectively prevent or treat viral infection after HSCT. Thus it would be desirable to develop T cells with the ability of simultaneously targeting AML relapse and viral infection. In this article, we now describe the generation of VST cells that are engineered to express CAR for a specific AML cell-surface antigen CD123 (CD123-CAR-VST). Using Dendritic cells (DCs) pulsed with EBV, Adv, and CMV peptides as sources of viral antigens, we generated VST from A2 donor peripheral mononuclear cells (PBMC). VST were then transduced with retroviral vector encoding D123-CAR to generate CD123-CAR-VST. We demonstrated that CD123-CAR-VST recognized EBV, Adv, and CMV epitopes and had HLA-restricted virus-specific cytotoxic effector function against EBV target. In addition, CD123-CAR-VST retained the specificity against CD123-positive AML cell lines such as MOLM13 and THP-1 in vitro. Thus our results suggested that CD123-CAR-VST might be a valuable candidate to simultaneously prevent or treat relapse and viral infection in AML HSCT recipients.
Poster

FOLLICULAR LYMPHOMA IN THE ERA OF TARGETED THERAPY

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Follicular lymphoma (FL) is composed of cells arranged in follicles which stem from germinal centers (1). FL typically express the antigens CD19, CD20, CD22, and CD79A. Most cases of FL are also positive for Bcl2, Bcl6, CD10, and the translocation t(14;18) which lead to continuous BCL2 protein expression and induce other critical events in the development of FL (2). CVP was used as first-line treatment and induced response rates (RR) between 60% and 80%, with a PFS of less than 2 years (3). Rituximab chemotherapy was determined to be a better alternative to Fludarabine and anthracycline-based therapies with RR of 81% and a PFS of 32 months (4; 5). The PRIMA study revealed the necessity to maintain anti-CD20 antibodies therapy. Alternate types of CD20 antibodies were not proven to be more effective. The combination of Lenalidomide and Rituximab (RR2) increases the ORR between 83.3% and 90% (7;8).

Treatments for relapsing/refractory FL are currently being investigated. However, population in early relapse after chemoimmunotherapy and for whom high-dose chemotherapy is muted could benefit of allogeneic transplantation (9). Relapse risk is higher for auto-HCT compared to allo-HCT with respective of PFS value 41% versus 58% at 5 years. The PFS is improved by 12.5 months after B-cell signaling pathway inhibitors (10;11), 14.9 months after Velcade and Rituximab (12), 20 months after Histone deacetylase inhibitors (HDAC) (13), 24 months after R2, and 27 months after Anti-PD1-blocking antibody (14). In the era of targeted therapy, given the length of the different PFS, the allograft in the FL should be considered even in severe forms.
BCL2 translocation is a specific marker of germinal center phenotype in double hit lymphomas

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Double hit lymphomas (DHL) are B-cell lymphomas with a MYC/8q24 rearrangement plus a BCL2/18q21 or BCL6/3q27 rearrangement or both. The pathologic diagnosis is usually high grade B-cell lymphoma or diffuse large B-cell lymphoma, and the prognosis is very poor. Although DHL usually arise in B-cell lymphomas of germinal center (GC) origin, which is routinely defined by immunohistochemistry for CD10, BCL6, and MUM1 in clinical cases, the relationship between CD10, BCL6 and MUM1 expression and translocation status in DHL has not been described.

Immunoperoxidase studies using antibodies directed against CD10, BCL6 and MUM-1 were performed on paraffin sections of B-cell lymphomas that were identified as DHL based on previous interphase FISH (fluorescence in situ hybridization) studies. The cut-off value for positivity was 30% for all antibodies tested. All patients consented to research use of their tissue.

Of the 55 DHL, 33 (60%) had MYC and BCL2 translocations (MYC/BCL2), 10 (18%) had MYC and BCL6 translocations (MYC/BCL6), and 12 (22%) had all three (MYC/BCL2/BCL6). Per the Hans algorithm, 49 (89%) were of GC phenotype (CD10+ [n=44] or CD10-/BCL6+/MUM1- [n=5]) and 6 (11%) were of non-GC phenotype (CD10-/BCL6+/MUM1+ [n=4] or CD10-/BCL6-/MUM1+ [n=1] or CD10-/BCL6-/MUM1- [n=1]). All 45 BCL2 translocation-positive DHL cases were of GC phenotype. Of the 10 BCL2 translocation-negative DHL, 4 (40%) were GC phenotype and 6 (60%) were non-GC phenotype.

The presence of a BCL2 translocation is a highly specific marker of GC phenotype in DHL. Conversely, DHL that lack a BCL2 translocation (MYC/BCL6) may be of GC or non-GC phenotype.
Poster

Prognostic significance of positron emission tomography prior and after autologous stem cell transplant for chemoresistant lymphoma.

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Aim

Evaluation of pre- and post-transplant PET scans prognostic significance.

Patients and methods.

Our retrospective analysis includes the data of 111 patients with high-risk HD (n=74, 67%) or NHL (n=37, 33%) receiving HDCT with auto-HSCT. Median age was 26 (10-62) years. HDCT regimens included BEAM (n=71), Bendamustine-EAM (n=34), CBV (n=3), other (n=3). Median follow-up is 28 (1-82) months. Pre-transplant PET scan (PET1) was performed in all pts. In 76 cases an additional PET scan was performed 2-3 months after auto-HSCT. Survival was estimated using Kaplan-Meier method.

Results

Initially 46% (n=52) were PET1-negative and 54% (n=59) were PET1-positive. Post-HDCT 68% (n=52) of pts were PET2-negative and 32% (n=24) PET2-positive (in 83% of cases these pts were PET1-positive). Fifty percent (n=26) of PET1-positive pts became PET2-negative, 7% (n=4) of PET1-negative pts were PET2-positive.

Two-year overall survival (OS) and event-free survival (EFS) were 76.5% and 72%, respectively. 2-year OS in PET1+ and PET1- patients were 62% and 89% (p=0.001), EFS were 61% and 85% (p=0.002), respectively. Two-year OS in PET2+ and PET2- pts were 56% and 96% (p=0.007), EFS were 62% and 92% (p=0.001), respectively. The prognosis of PET1+PET2- group didn’t differ significantly from the one in PET1- patients. All 4 patients with new lesions on PET2 are currently alive with no signs of disease progression.

Conclusion

While overall prognosis of PET-positive pts is significantly worse, an additional post-transplant PET can be used for further risk stratification. The PET1+PET2+ group is most at risk for relapse. Therefore, these patients could be candidates for preemptive therapy.
Identification of clonal evolution and adverse cytogenetic abnormalities using iFISH in a prospective series of 138 newly diagnosed multiple myeloma

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Background. Conventional cytogenetics is no longer the appropriate technique to detect cytogenetic markers in multiple myeloma (MM). Low plasma cells proliferative index in culture hampered efficient mitotic collection, only 35% of patients presented abnormal caryotypes often associated with advanced stage disease. Interphasic In-situ hybridization (iFISH) performed on CD138 sorted plasma cells (PC+) allow to detect abnormalities independently of proliferative index.

Methods. Between January 2014 and June 2015, we investigated 138 patients with MM using iFISH targeting 4 unfavorable cytogenetic abnormalities: TP53 deletion, t(4;14) IGH-FGFR3 translocation, 1p32-CDKN2C deletion and 1q21-CKS1B gain. Thresholds for each probe were determined on PC+ from patients without MM.

Results. iFISH has identified abnormalities in 79.7% of patients with a median of 2 abnormalities per case [0 to 4.4]. We detected adverse cytogenetic markers in 48.1% of cases, TP53 deletion in 14.6%, t(4;14) IGH-FGFR3 in 13.0%, 1q21 gain in 32.4% and 1p32 deletion in 11.0%. We observed a combination of 2 or 3 unfavorable markers in 32.6% of cases. There was preferential association between t(4;14) and 1q21 gain (p=.004). Moreover, iFISH allowed to detect clonal heterogeneity in 35.3% of patients at diagnosis, 80.8% presented with at least one adverse FISH marker. The median number of related clones was significantly higher when a 1q21 gain was identified (median = 2, p.0001).

Conclusion. iFISH allowed to detect 16% of high risk patients with combination of 2 or more adverse cytogenetic markers. iFISH confirmed that clonal evolution was an early event in MM pathogenesis, mostly involving 1q21 gain.
Flow Cytometric and Lymphomas

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Flow cytometry has become an important tool in the diagnosis and characterization of hematologic and lymphoid neoplasia. Multiparametric flow cytometry method (FCM) is a powerful diagnostic tool that permits rapid assessment of cellular antigen expression to quickly provide immunophenotypic information suitable for disease classification. This technology serves as an excellent complement to microscope-based traditional diagnostic methods and adds distinctive capabilities that are unmatched by any other diagnostic methods. Flow cytometry is ideal for fluids where cells are naturally suspended but is also useful in lymphoid tissues, from which single-cell suspensions can be easily obtained. The advantages of flow cytometry are largely based on its ability to analyze very rapidly, even in small samples, multiple cell properties simultaneously, including size, granularity, surface and intracellular antigens, and DNA content. The quantitative nature of the data produced, both with regard to cell population distributions and to expression of individual cell antigens, offers objective criteria for interpretation of results. Examples of applications include the detection of clonal cells in B-cell lymphoma, the recognition of antigenic expression anomalies in B- or T-cell malignancies, the identification of malignant plasma cells, and the rapid measurement of cell cycle fractions. The unique attributes of flow cytometry allow for increased sensitivity in the detection of neoplastic cells and should contribute to improving accuracy and precision in the diagnosis and classification of lymphomas and lymphoproliferative disorders. We highlight an overview of procedures for processing and immunophenotyping non-Hodgkin B- and T-cell lymphomas and also describe our strategy for the sensitive and specific diagnosis of classical Hodgkin lymphoma. With all the uses and advantages that FCM offers, it is equally important to acknowledge the limitations of this technique as well. One of the main drawbacks of immunophenotyping by FCM is the requirement for fresh, unfixed tissue for analysis. The applications of FCM and IHC in the diagnosis and classification of lymphomas can both be complementary and competitive.
An autoantibody to platelet glycoprotein (GP) IIb/IIIa was produced in a 27 year old woman who had a previous history of the malignant lymphoma of the stomach. The aggregations of the patient’s platelets showed losses of the primary waves in response to ADP and marked hypoaggregation in response to collagen, while agglutination by ristocetin was normal. Crossed immuno-electrophoresis (CIE) of her platelets solubilized by 1% Triton X-100 revealed an abnormal biphasic precipitate line of GP IIb/IIIa complex. Nine months later, she developed severe thrombocytopenia along with a relapse of the lymphoma in the cervical lymph nodes. The patient’s IgG, immunoglobulin G2 (IgG2) subclass which was collected during her thrombocytopenic period and purified, inhibited ADP-, epinephrine- and collagen-induced aggregations of normal platelets. In CIE, the 125I-labelled IgG of the patient, inserted into the intermediate gel, was incorporated into the precipitation line of the GP IIb/IIIa complex of normal platelets. Radiation treatment to the cervical lymph nodes dramatically normalized both the function and the count of the patient’s platelets. From these findings, it is suggested that an autoantibody to the GP IIb and/or IIIa was produced by the lymphoma cells.
The frequency of genetic anomalies according to clonal plasma cells’ phenotype in patients with newly diagnosed (ND) multiple myeloma (MM).

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Background. Genetic anomalies (GA) are primary link of pathogenesis in MM. GA lead to formation of clonal plasma cells, which has different phenotype.

Aim. To estimate the incidence of GA and their correlation with clonal plasma cells’ phenotype in patients with ND MM.

Methods. We analysed 22 patients with ND MM (median age 57 years, range 38-80; male/female – 1:1.75). Cytogenetic analysis was performed on bone marrow samples using standard GTG-method. Metaphase FISH analysis was performed according to the manufacturer’s protocol using DNA probes: LSI 13(RB1)13q14, IGH/CCND1, IGH/FGFR3, LSI TP53 (17q13.1). 8-color immunophenotypic by flow cytometry using antibody to CD45, CD38, CD138, CD56, CD19, CD20, CD27 and CD117 antigens.

Results. Translocation t(11;14) was detected in 3/14 (21.4%) patients, del(13q) – 2/14 (14.3%), t(11;14) – 3/14 (21.4%), hypodiploidy – 1/20 (5%), del(17p) – 0% patients. Clonal plasma cells’ phenotype CD38+CD138+CD45– was detected in 100%. Expression CD56+ was revealed in 11/22 (50%) patients, CD19+ in 9/22 (40.9%), CD117+ in 5/22 (22.7%), CD20+ in 1/22 (4.5%), CD27+ in 1/22 (4.5%). The frequency of GA didn’t depend on clonal plasma cells’ phenotype and was 27.3%(3/11) in CD56+ phenotype, 23.8%(5/21) – CD20–, 23.8%(5/21) – CD27–, 23.5%(4/17) – CD117–, 23%(3/13) – CD19–, 22.2%(2/9) – CD19+, 20%(1/5) – CD117+, 18.2%(2/11) – CD56–, 0%(0/1) – CD20+, 0%(0/1) - in CD27+ phenotype. Patients of standard risk group according to mSMART 2.0 with GA had CD19-negative plasma cells’ phenotype vs. CD19-positive phenotype in patients of intermediate and high-risk groups (p<0.05). 3-years overall survival in standard risk group with CD19– phenotype was 92,3%, CD19+ – 77,7% (p<0.05).

Conclusion. Identification of GA, which has adverse forecast, correlates with CD19+ plasma cells phenotype. The combined definition of plasma cells phenotype and GA can improve the system of risk stratification in MM.
Poster

The frequency of occurrence of minimal residual disease (MRD) into different prognostic groups of patients with chronic lymphocytic leukemia (CLL).

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Background. It is known, that genetic factors and the absence of MRD are strongly affecting prognosis of CLL.

Aim. To determine the influence of genetic abnormalities (GA) on achieving MRD-negative remissions in patients with CLL.

Methods. Twenty-four pts (median age 57 years, range 35-67; male 14, female 10) with newly diagnosed CLL were included. The CLL was diagnosed according to the standard basic examination. Cytogenetic studies were performed on blood samples using standard GTG-method. Interphase FISH analyses were performed according to the manufacturer’s protocol using DNA probes. We have used NCI revised guidelines for treatment initiation and assessment of response. All patients treated subsequently with rituximab maintenance. MRD was detected by multicolor flow cytometry.

Results. The frequency of GA was 50.0% (12/24): 15.0% (3/20) – by conventional karyotyping, 47.8% (11/23) – by FISH analyses and 9.5% (2/21) – using both methods. Stratification of patients into prognostic groups based on identified GA. Favorable prognosis (Group1) - del(13q) (n = 5); neutral (Group2) - normal karyotype (n = 12) or trisomy 12 (n = 3); unfavorable (Group3) - del(11q) (n = 3) or the complex karyotype (n = 1). Statistically significant differences in the frequency of achieving MRD-negative remissions between FCR (5/11) vs. RB (5/13) were not detected (p>0.05). Complete remission (CR) was reached in 37.5% (9/24) pts, partial remission – 62.5% (15/24). The MRD-negative - in 10 patients: in Group1 – 2/5 (40%; CR – 1), in Group2 – 5/15 (33.3%, CR – 6), in Group3 – 75.0% (3/4; CR – 2). Statistically significant differences in PFS were detected between MRD-negative and MRD-positive groups (p=0.03). Median of PFS in MRD-negative has not been reached, in MRD-positive - 33.1 month.

Conclusions. Further researches aimed at examining the relationship between the presence or absence of MRD and genetic prognostic groups, will help to understand the most important factors affecting the OS and PFS.
Rearrangement of cMyc fined by FISH in patients with DBCL all treted with RCHOP, what that mean?

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Introduction: Diffuse large B-cell lymphoma accounts for 30% of all lymphoid malignancies. The translocation t(8;14)(q24;q23), cMyc gene was the first translocation detected in lymphoid neoplasms. In unselected DBCL series, rearrangements of the MYC gene were discovered in approximately 5 to 10% of cases. Material and methods: We studied 62 patients with de novo DBCL. Treatment consisted of R-CHOP/21 day. We used standard processing methods for Fluorescent in situ hybridization (FISH) analysis performed for c-Myc. Statistical analysis was performed by (SPSS 22.0), significance of p<0.05. Aims of study: This study analysis frequencies of cMyc in different outcome group. We analysis the prognostic relevance of cMyc in patients with differente IPI, outcome, OS and refracterio disease. Results: We fined 62/27 cMyc+ patients. In both analyzed groups (died vs live/ 57,6% vs 62,1%) no diferente (χ² test, p=0.719). In the groups with cMyc – vs cMyc+ , 44% vs 42,5% was survival after 84 months. There is no difference in OS between cMyc+ vs cMyc- patients in group with low IPI (Log Rank test ,p = 0,798), or in the group with high IPI risk (Log Rank test ,p =0,336). No significant difference regarding the occurrence cMyc + vs cMyc- in refracterio disease (χ² test ,p=0,800). Conclusion: We had patients with high frequents of cMyc , but high IPI score was independent risk factor for poor outcome. Refracterio disease is problem too, but something else is maybe associated with cMyc. Perhaps the solution is large studies for cMyc+ DBCL.

Key words: DBCL, FISH, cMyc, OS, refracterio disease
Use of Hevylite in the diagnosis and follow-up of monoclonal proteins difficult to detect by SPEP

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Introduction

Serum protein electrophoresis (SPEP) is technique for identification monoclonal protein (MP), with low sensitivity for MP below 1g/L. IgA MP often migrate in SPE beta region along with other proteins (transferrin, β-2 microglobulin), which masks identification and precise quantification of MP. Electrophoretic immunofixation (IFX) is qualitative technique that is dependent of observer expertise. Total immunoglobulin not differentiate MP from polyclonal component. Hevylite (HLC) allows the separate quantification of heavy/light pairs of immunoglobulins allowing quantification of involved monoclonal immunoglobulin. HLC ratio will also indicate presence of monoclonal process.

Objective

Evaluate utility Hevylite as complementary assay to traditional techniques used in diagnosis and follow-up of MP.

Material & methods

59 patients with suspected MP for unexplained increase of beta migrating region. SPEP, IFX (SEBIA), Total Igs (SIEMENS), Hevylite (The Binding Site).

Results

44% patients presented an IgA MP, confirmed by IFX and altered Hevylite ratio that indicates monoclonality: 0.55 (0.82-2.04). Median value involved HLC and total IgA were 5.32g/L and 6.095g/L, respectively
Follow-up of MMigA: Patient diagnosed MMigAk in 2012. August 2014 complete remission. June 2015 HLC ratio pathological 2.67g/L, K/L 5.09 mg/L, while IFX SPEP IgAT were and not pathologicals.

Conclusions

Detection of small MP by SPEP requires an experienced professional due to low resolution and subjectivity of the technique.
Hevylite has allowed better identification and quantification of MP that were difficult to visualize by SPEP and IFX is not quantitative technique
Hevylite in follow-up of an IgA MM patient allowed identification of residual disease undetected by IFX and SPEP.
IGHV-IGHD-IGHJ REARRANGEMENTS IN MACEDONIAN CLL PATIENTS

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Abstract: Introduction: B-cell chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease with many patients surviving for decades with watch and wait strategy or no treatment, whereas others surrender to their disease despite therapy. In recent years, new molecular prognostic factors came to light that have significantly improved the stratification of the CLL patients. One of the most important molecular predictors, the immunoglobulin VH gene mutational status, divides CLL into two prognostic groups, depending on the presence or absence of somatic hypermutation, where unmutated U-CLL are associated with remarkably worse prognosis than mutated U-CLL.

The aim of the study was evaluation of rearrangement of IG genes profile in Macedonian CLL patients in line with facts that there are some geographic linked variations in IG genes.

Material and methods: In this study, mutational status and configuration of IGHV-IGHD-IGHJ rearrangements in 70 treatment naïve CLL patients were analyzed using reverse transcriptase–polymerase chain reaction (RT-PCR) and sequencing methodology at the Center for Biomolecular Pharmaceutical Analyses, Faculty of Pharmacy, Skopje, Macedonia.

Results: Our evaluation have shown that 52.8% patients belonged to the U-CLL subset, whereas 47.1% belonged to the M-CLL subset. The most frequently expressed IGHV subgroup was IGHV3 (41.4%), followed by IGHV1 and IGHV4 (28.5%), IGHV5 (1.4%). In the IGHD and IGHJ sets most frequently expressed was IGHD3 (55.7%) IGHJ6 gene (37.1%) respectively.

Conclusion: Our evaluation of mutational status on IGVH, IGDH, and IGJH gene in Macedonian CLL patients, resulted with data which are consubstantial to those from Mediterranean area and west Balkan.
BCL-2 INHIBITION SENSITISES BCL-2-DYSREGULATED / OVEREXPRESSING LYMPHOMA TO THE PRO-APOPTOTIC ACTION OF THE SELECTIVE SEROTONIN REUPTAKE INHIBITOR FLUOXETINE

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BACKGROUND: We previously identified compounds in the SSRI class of antidepressants as being anti-proliferative and pro-apoptotic to the constituent neoplastic cells of Burkitt’s lymphoma (BL). Efficacy was deemed achievable within conventional therapeutic doses. A wider range of B-cell malignancy revealed that while broad anti-proliferative action from the SSRI remained, apoptosis was thwarted in the face of Bcl-2-dysregulation / over-expression. Here we have explored the consequence of Bcl-2 inhibition on the pro-apoptotic potential of the SSRI fluoxetine in scenarios where Bcl-2 is dysregulated / over-expressed. METHODOLOGY: To illustrate the principle, the following cell lines were selected for study: (i) DoHH2, derived from a case of t(14;18) diffuse large B-cell lymphoma (thereby expressing dysregulated Bcl-2 off the Ig HC/bcl-2 translocation); (ii) KHM2B, derived from a case of BL carrying the hallmark t(8;14) myc/Ig HC translocation plus in addition (and unusually) the bcl-2-dysregulating t(14:18) translocation; (iii) L3055/Bcl-2, BL cells engineered to constitutively over-express a bcl-2 transgene to a (protein) level approximately 5-times that of DoHH2. Apoptosis was followed between 48-96 hours using propidium iodide with an active caspase-3 stain to allow discrimination between viable, early apoptotic, late apoptotic, and necrotic cells. RESULTS: In all three cell lines, fluoxetine – while clearly anti-proliferative – failed to deliver apoptosis, either early or late, with full viability maintained throughout culture. Where a Bcl-2 family inhibitor was included, each of the three cell lines now succumbed to fluoxetine’s pro-apoptotic drive. Applying optimal synergistic combinations of the two compounds could result in 95% apoptosis of otherwise refractory cells. CONCLUSION: A dual therapy modality combining a widely used SSRI with a proven Bcl-2 inhibitor becomes, from the data presented here, a promising option in B-cell tumors otherwise refractory to fluoxetine’s pro-apoptotic promise. As fluoxetine accumulates some 20-fold in brain over blood, this option could be particularly attractive in primary CNS lymphoma.
The T cell immunoglobulin and mucin domain 3 (Tim-3) is a plasma membrane-associated immune receptor which is involved in several types of biological responses of human immune cells of both lymphoid and myeloid lineages. It is highly expressed in most acute myeloid leukaemia (AML) cells and is therefore currently considered as a possible target for AML therapy. Tim-3 has also been found to negatively regulate anti-tumour immunity because of its contribution to T cell exhaustion in cancer. However, biochemical and physiological functions of Tim-3 remain unclear and form the main aim of our work.

We found that Tim-3 protein expression is significantly higher in primary human AML cells compared to primary healthy human leukocytes. Interestingly, in healthy leukocytes Tim-3 protein was mainly present inside the cells, while malignant cells had it mostly on their surface. We discovered that Tim-3 mediates a significant upregulation of the mTOR pathway in a ligand-induced manner. Agonistic antibody and galectin-9 (one of the Tim-3 natural ligands) were used to trigger Tim-3 activity. The mTOR upregulatory effect described above was in line with activation of hypoxia-inducible factor 1 (HIF-1) transcription complex and secretion of vascular endothelial growth factor (VEGF) as well as tumour necrosis factor alpha (TNF-α). Similar effects were observed in primary human healthy leukocytes [1, 2].

Furthermore, we are currently looking at biochemical mechanisms underlying high levels of Tim-3 expression in malignant hematopoietic cells and its involvement in employment of healthy leukocytes and somatic tissues in leukaemia progression. These results will be presented in the poster.

References
